AMENDMENTS TO THE SPECIFICATION

Please amend the paragraph beginning at page 1, line 20, as follows:

In an approach of post-genomic and physiomic, it was established the molecular and functional bases providing evidence for the existence in mammals of a hormonal messenger of the intercellular communication, i.e., the final mature peptide generated from SMR1 pre-prohormone: SMR1-Pentapeptide, named today Sialorphin (of sequence QHNPR (SEQ ID NO: 8)). Hence, sialorphin is an exocrine and endocrine peptide-signal, whose expression is under activational androgenic regulation and secretion is evoked under adrenergic-mediated response to environmental stress, in male rat (Rougeot et al., 1997).

Please amend the paragraph beginning at page 4, line 23, as follows:

Sialorphin is the only identified physiological systemically active regulator of the membrane-bound enkephalinase activity in mammals. This raises the question of the existence of such endogenous NEP-ectopeptidase inhibitor in human saliva and blood. No immunoreactive QHNPR peptide (SEQ ID NO: 8) (sialorphin) was detected in male human saliva using highly sensitive and specific radioimmunoassay (Rougeot et al., 1994). However, bibliographical data let suppose the presence of low molecular weight substances ($\leq$3000 Da), inhibiting the NEP ectopeptidase activity in human, notably in the human saliva. Although this(ese) salivary component(s) was(were) not biochemically characterized, a gender-related difference was observed in the salivary production of this(ese) inhibitor(s) of human enkephalin-degrading ectoenzymes (Marini and Roda, 2000). Strikingly, the situation is very similar to that one identified by the inventors in male rat, wherein the submandibular gland and the saliva represented the compartments of major synthesis and secretion of sialorphin, respectively.

Please amend the paragraph beginning at page 5, line 24, as follows:

The numerous data collected by the inventors support that the new peptide, of sequence QRFSR (SEQ ID NO: 3), derives from the BPLP protein ("Basic Prolin-rich Lacrimal Protein").

Please amend the paragraph beginning at page 6, line 8, as follows:

At such consensus sites, the inventors then found out a sequence QRFSR (SEQ ID NO: 3), structurally closely related to that of rat QHNPR sialorphin (SEQ ID NO: 8).

Please amend the paragraph beginning at page 6, line 16, as follows:

More particularly, the present invention is drawn to maturation products of the BPLP protein, in particular the QRFSR peptide (SEQ ID NO: 3), as well as peptide derivatives and mimetics thereof, useful to potentialise the effects of neuroendocrine peptide messengers which control the nociceptive transmission (e.g. enkephalins), the well-being and/or the homeostatic exchanges of Na/Pi/Ca/H2O mainly (e.g. natriuretic peptides).

Please amend the paragraph beginning at page 9, line 3, as follows:

The peptides of the invention include peptides or peptide derivatives which comprise, consist essentially of or consist of sequence X1-X2-Arg-Phe-Ser-Arg (SEQ ID NO: 6), wherein X1 represents H, ~~atom or a~~ Tyr, or Cys ~~aminoacid~~, X2 represents Gln or Glp when X1 is H, or X2 represents Gln when X1 is Tyr or Cys. When the peptide of the invention comprises or consists essentially of sequence X1-X2-Arg-Phe-Ser-Arg (SEQ ID NO: 6), said sequence is the C-terminal part of the peptide of the invention.

Please amend the paragraph beginning at page 9, line 9, as follows:

Preferred peptides according to the invention comprise, consist essentially of, or consist of sequence QRFSR (SEQ ID NO: 3).

Please amend the paragraph beginning at page 9, line 15, as follows:

Still another peptide of the invention is the peptide that consists of sequence CQRFSR (SEQ ID NO: 5).

Please insert the following new paragraph on page 9, between lines 16 and 17:

Yet another peptide of the invention is the peptide that consists of sequence GlpRFSR (SEQ ID NO: 7).

Please amend the paragraph beginning at page 11, line 7, as follows:

The invention also relates to a molecular complex comprising :

- a metallo-ectopeptidase receptor, especially a NEP receptor or an APN receptor, especially a NEP receptor, binding site of the BPLP-protein or maturation products thereof, *e.g.* QRFSR (SEQ ID NO: 3);

- the BPLP-protein or maturation products thereof, *e.g.* QRFSR (SEQ ID NO: 3).

Please amend the paragraph beginning at page 11, line 20, as follows:

Particularly, the present invention provides nucleic acids coding for peptides which comprise, consist essentially of, or consist of sequence X1-X2-Arg-Phe-Ser-Arg (SEQ ID NO: 6) as above defined. When the peptide of the invention comprises or consists essentially of sequence X1-X2-Arg-Phe-Ser-Arg (SEQ ID NO: 6), said sequence is the C-terminal part of the peptide of the invention. In preferred embodiments, the present invention provides acid

nucleic coding for peptides which comprise, consist essentially of, or consist of sequence QRFSR (SEQ ID NO: 3). In a most preferred embodiment, the present invention provides a nucleic acid coding for QRFSR (SEQ ID NO: 3) or a nucleic acid coding for YQRFSR (SEQ ID NO: 4).

Please amend the paragraph beginning at page 16, line 1, as follows:

More particularly, the present invention provides antibodies directed against peptides which comprise, consist essentially of, or consist of sequence X1-X2-Arg-Phe-Ser-Arg (SEQ ID NO: 6) as above defined. When the peptide of the invention comprises or consists essentially of sequence X1-X2-Arg-Phe-Ser-Arg (SEQ ID NO: 6), said sequence is the C-terminal part of the peptide of the invention. In preferred embodiments, the present invention provides antibodies directed against (i.e. that specifically recognize) peptides which comprise, consist essentially of, or consist of sequence QRFSR (SEQ ID NO: 3). In a most preferred embodiment, the present invention provides antibodies directed against (i.e. that specifically recognize) QRFSR (SEQ ID NO: 3) or antibodies directed against (i.e. that specifically recognize) YQRFSR (SEQ ID NO: 4) or antibodies directed against (i.e. that specifically recognize) CQRFSR (SEQ ID NO: 5).

Please amend the paragraph beginning at page 18, line 1, as follows:

The present invention further relates to an in vitro method for diagnosis, prognosis or determination of the evolution of a condition involving an altered production (i.e. a decrease or an increase of production in comparison to a control subject) of BPLP or of any of its maturation products. The method comprises detecting, or quantifying in a biological sample of a test subject, a BPLP protein or maturation products thereof, especially QRFSR (SEQ ID NO: 3), compared with the same in a biological sample of a control subject.

Please amend the paragraph beginning at page 19, line 6, as follows:

Such assay methods comprise contacting a biological sample with a binding partner capable of selectively interacting with a BPLP protein or maturation products thereof, especially QRFSR (SEQ ID NO: 3), present in the sample. The binding partner is generally an antibody, that may be polyclonal or monoclonal, preferably monoclonal.

Please amend the paragraph beginning at page 19, line 14, as follows:

For example, the presence or production of BPLP protein or of any of its maturation products, or a mutated form of the protein or of the maturation product, can be detected by incubating a biological sample with an antibody that specifically recognizes the BPLP protein or an antibody that specifically recognizes a maturation product thereof, especially QRFSR (SEQ ID NO: 3), e.g. using standard electrophoretic and liquid or solid immunodiagnostic techniques, including immunoassays such as competition, direct reaction, or sandwich type assays. Such assays include, but are not limited to, Western blots; agglutination tests; enzyme-labeled and mediated immunoassays, such as ELISAs; biotin/avidin type assays; radioimmunoassay such as those using radioiodinated or tritiated BPLP protein or any of its maturation products, especially QRFSR (SEQ ID NO: 3); immunoelectrophoresis; immunoprecipitation, etc. The reactions generally include revealing labels such as fluorescent, chemiluminescent, radioactive, enzymatic labels or dye molecules, or other methods for detecting the formation of a complex between the antigen and the antibody or antibodies reacted therewith.

Please amend the paragraph beginning at page 19, line 29, as follows:

The aforementioned assays generally involve separation of unbound BPLP protein or unbound maturation products thereof, especially unbound QRFSR (SEQ ID NO: 3), from the

bound BPLP protein or maturation products, especially QHNPR (SEQ ID NO: 8), to the specific antibody which is immobilized on a solid phase. Solid supports which can be used in the practice of the invention include supports such as nitrocellulose (e.g., in membrane or microtiter well form); polyvinylchloride (e.g., sheets or microtiter wells); polystyrene latex (e.g., beads or microtiter plates); polyvinylidine fluoride; diazotized paper; nylon membranes; activated beads, magnetically responsive beads, and the like.

Please amend the paragraph beginning at page 20, line 5, as follows:

Thus, in one particular embodiment, the presence of bound BPLP protein or maturation products thereof, especially QRFSR (SEQ ID NO: 3), from a biological sample can be readily detected using a secondary binder comprising another antibody, that can be readily conjugated to a detectable enzyme label, such as horseradish peroxidase, alkaline phosphatase or urease, using methods known to those of skill in the art. An appropriate enzyme substrate is then used to generate a detectable signal, such as a chromogenic or fluorogenic signal for example. In other related embodiments, competitive-type ELISA techniques can be practiced using methods known to those skilled in the art.

Please amend the paragraph beginning at page 31, line 6, as follows:

The invention further relates to the use of an agent that modulates the interaction between endogenous BPLP protein or maturation product, *e.g.* QRFSR (SEQ ID NO: 3), and a membrane metallopeptidase for the preparation of a therapeutic composition for preventing or treating diseases wherein a modulation of the activity of said membrane metallopeptidase is sought.

Please amend the paragraph beginning at page 31, line 13, as follows:

The methods that allow a person skilled in the art to select and purify candidate compounds that bind to the same targets and have an agonist or an antagonist biological activity of the BPLP protein or maturation products thereof, *e.g.* the QRFSR peptide (SEQ ID NO: 3), are described hereunder.

Please amend the paragraph beginning at page 31, line 21, as follows:

The invention provides an *in vitro* method for screening compounds for their ability to bind to the NEP binding site for the BPLP protein or a maturation product thereof, *e.g.* the QRFSR peptide (SEQ ID NO: 3), comprising the steps of:

a) incubating a candidate compound with a NEP expressing cell, in the presence of the BPLP protein or a maturation product thereof, *e.g.* the QRFSR peptide (SEQ ID NO: 3), or any peptide retaining the binding specificity or the physiological activity of BPLP protein or of its maturation products, *e.g.* the peptide YQRFSR (SEQ ID NO: 4);

b) determining the ability of the candidate compound to compete with the BPLP protein or a maturation product thereof, *e.g.* the QRFSR peptide (SEQ ID NO: 3) or with the peptide retaining the binding specificity or the physiological activity of BPLP protein or of its maturation products, *e.g.* the peptide YQRFSR (SEQ ID NO: 4), for binding to NEP.

Please amend the paragraph beginning at page 32, line 3, as follows:

The NEP expressing cell may be in a cell culture, such as a confluent target cell culture monolayer, or a target organ specimen or a tissue sample (e.g. cryosections, slices, membrane preparations or crude homogenates) that contains NEP binding sites for the BPLP protein or a maturation product thereof, *e.g.* the QRFSR peptide (SEQ ID NO: 3).

Please amend the paragraph beginning at page 32, line 11, as follows:

Other preferred tissue samples that can be used in the screening methods according to the present invention are all peripheral tissue preparations that are known to be enriched in NEP-peptidase and/or to be targets for the BPLP protein or a maturation product thereof, e.g. the QRFSR peptide (SEQ ID NO: 3). For example one may use mammal renal outer medulla, placenta, testis, prostate and bone. For example, such a procedure can be applied to tissues and/or cells of mouse, rat or human origin or cell lines transfected with metallo-ectopeptidase cDNA, in particular NEP cDNA, especially human NEP cDNA.

Please amend the paragraph beginning at page 33, line 13, as follows:

In said above process, a half saturating concentration is the concentration of the labeled BPLP protein or maturation product thereof, *e.g.* the QRFSR peptide (SEQ ID NO: 3) (or the peptide that retains the binding specificity or the physiological activity of the BPLP protein or of its matured products) which binds 50 % of the NEP binding sites.

Please amend the paragraph beginning at page 33, line 18, as follows:

This process also allows to define the relative affinity of the candidate compound compared to the BPLP protein, or maturation products, *e.g.* QRFSR (SEQ ID NO: 3) affinity (or the peptide that retains the binding specificity or the physiological activity of the BPLP protein or of its matured products).

Please amend the paragraph beginning at page 34, line 17, as follows:

One may further compare the affinity of each candidate compound quantified to the one of the other candidate compounds, so that the relative affinity of candidate compound

that specifically binds to the NEP binding site for the BPLP protein or a maturation product thereof, *e.g.* the QRFSR peptide (SEQ ID NO: 3), is determined.

Please amend the paragraph beginning at page 34, line 25, as follows:

a) incubating a candidate compound with a NEP expressing cell, in the presence of (i) the BPLP protein or a maturation product thereof, *e.g.* the QRFSR peptide (SEQ ID NO: 3), or any peptide retaining the binding specificity or the physiological activity of the BPLP protein or of its matured products, and (ii) a NEP substrate ;

Please amend the paragraph beginning at page 37, line 3, as follows:

Figure 1 shows representative cation-exchange HPLC profile of ^{3}H-YQRFSR (SEQ ID NO: 4) marker added to 2.5 ml salivary methanol-acid extract corresponding to 2.5 ml human saliva. The recovery of the major radioactive peak was evaluated at 75-84 % (dotted bars).

Please amend the paragraph beginning at page 37, line 19, as follows:

Figure 5 shows the effect of BPLP-QRFSR peptide (SEQ ID NO: 3) on the breakdown of substance P by human ecto-endopeptidase activity (LNCaP cell line), the effective concentration of QRFSR peptide (SEQ ID NO: 3) ranged from 1 to 25 μM and being half-maximal at 11 μM.

Please amend the paragraph beginning at page 37, line 23, as follows:

Figure 6 shows the effect of YQRFSR (SEQ ID NO: 4) derivative of hBPLP-QRFSR peptide (SEQ ID NO: 3) on the breakdown of substance P by human ecto-endopeptidase

activity (LNCaP cell line), the effective concentration of YQRFSR peptide (SEQ ID NO: 4) ranged from 5 to 50 μM and being half-maximal at 30 μM.

Please amend the paragraph beginning at page 37, line 27, as follows:

Figure 7 shows the effect of YQRFSR (SEQ ID NO: 4) derivative of hBPLP-QRFSR peptide (SEQ ID NO: 3) on the breakdown of substance P by rat NEP ecto-endopeptidase activity (renal tissue), the effective concentration of YQRFSR peptide (SEQ ID NO: 4) ranged from 5 to 75 μM and being half-maximal at 38 μM.

Please amend the paragraph beginning at page 37, line 31, as follows:

Figure 8 is a RP-HPLC chromatographic analysis of the YQRFSR peptide (SEQ ID NO: 4). The YQRFSR peptide (SEQ ID NO: 4) (175 μM) was not metabolized by human cell surface endopeptidases, *in vitro*, whilst it inhibited by 70% the substance P endoproteolysis mediated by human NEP ectoendopeptidase. The RP-HPLC chromatographic characteristics revealed that :

1/ the YQRFSR peptide (SEQ ID NO: 4) is not metabolized by human cell membranes containing NEP; 93 % was recovered as intact peptide against 94 % in absence of metabolizing membranes;

2/ in the same experimental conditions the YQRFSR peptide (SEQ ID NO: 4) inhibits by 70% the endoproteolysis of substance P by these human cell membranes.

Please amend the paragraph beginning at page 38, line 8, as follows:

Figure 9 shows the inhibitory effect of QRFSR-peptide (SEQ ID NO: 3) on the breakdown of substance P by recombinant human NEP. Concentration-dependent inhibitory effect of QRFSR-Peptide (SEQ ID NO: 3) on soluble recombinant human NEP activity and

no effect of QRFSR-peptide (SEQ ID NO: 3) on the endoproteolysis of substance P by soluble recombinant hDPPIV activity.

Please amend the paragraph beginning at page 38, line 13, as follows:

Figure 10 shows the inhibitory effect of QRFSR-peptide (SEQ ID NO: 3) on the breakdown of APN synthetic substrate by cell surface human APN. Concentration-dependent inhibition by QRFSR-peptide (SEQ ID NO: 3) of the cleavage of Ala-pNA chromogenic substrate by cell surface HEK- hAPN.

Please amend the paragraph beginning at page 38, line 17, as follows:

Figure 11 shows the inhibitory effect of QRFSR-peptide (SEQ ID NO: 3) on the breakdown of NEP synthetic substrate by cell surface human NEP. Concentration-dependent inhibition by QRFSR-peptide (SEQ ID NO: 3) of the cleavage of Mca-BK2 fluorogenic substrate by cell surface HEK- hNEP.

Please amend the paragraph beginning at page 38, line 21, as follows:

Figure 12 shows the *in vivo* effect of YQRFSR-peptide (SEQ ID NO: 4) on the time spent by rat in paw licking of the formalin-injected hind paw; Mean ± SEM.

Please amend the paragraph beginning at page 38, line 23, as follows:

Figure 13 shows the *in vivo* effect of YQRFSR-peptide (SEQ ID NO: 4) on the number of pain spasms following hind paw formalin injection; Mean ± SEM.

Please amend the paragraph beginning at page 38, line 25, as follows:

Figure 14 shows the *in vivo* effect of YQRFSR-peptide (SEQ ID NO: 4) on the index of pain spasms during the 60 minute post injection of formalin. The analgesia induced by QRFSR-derived peptide (SEQ ID NO: 3) requires the activation of endogenous opioid receptors.

Please amend the paragraph beginning at page 41, line 2, as follows:

- Mca-R-P-P-G-F-S-A-F-K-(Dnp)-OH (SEQ ID NO: 12) and/or Suc-A-A-F-Amc (SEQ ID NO: 13) (NEP) (R&D systems and Bachem)

Please amend the paragraph beginning at page 41, line 6, as follows:

- Modified tritiated substance P [(3,4^{3}H)Pro^{2}-Sar^{9}-Met(O_2)^{11}]-Substance P (DuPont-NEN) and Native Substance P: R-P-K-P-Q-Q-F-F-G-L-M (SEQ ID NO: 14) (NEP-DPPIV-ACE) (Peninsula-Biovalley)

Please amend the paragraph beginning at page 41, line 9, as follows:

- Native Met-enkephalin: Y-G-G-F-M (SEQ ID NO: 15) (NEP-APN) (Peninsula-Biovalley) Measuring the hydrolysis of these substrates by cell-membrane peptidases in the presence and absence of different available selective synthetic peptidase inhibitors assessed the specificity of the peptidase assay:

Please amend the paragraph beginning at page 44, line 29, as follows:

- Ciphergen ProteinChip and amino-acid sequence analyses. N-terminal sequence analysis was performed by automated Edman degradation using Applied Biosystems peptide sequanators (plate-forme d'Analyse et de Microséquençage des Protéines, Institut Pasteur).

The molecular form eluting from the ultimate RP-HPLC at 18 min-retention time (fraction 20) corresponded to 690 and 769.5 Da molecular mass and to the following sequence of five amino acid residues: QRFSR (SEQ ID NO: 3). That one eluting at 26 min-retention time (fraction 28) corresponded to two molecular components of 622-666 Da and 6495 Da, respectively; the amino-acid determination of the highest molecular mass indicated that it corresponds to a salivary Basic Proline-Rich Polypeptide sequence, the human PRP-E of 61 amino-acid sequence (Isemura et al., 1982).

Please amend the paragraph beginning at page 45, line 8, as follows:

By analogy with the rat salivary sialorphin, these data provide direct evidence for the existence of a human salivary sialorphin-like, a QRFSR pentapeptide (SEQ ID NO: 3) of structure and function closely related to those of rat QHNPR pentapeptide (SEQ ID NO: 8) and which is secreted into the human salivary secretions; they support that QRFSR (SEQ ID NO: 3) is the mature product proteolytically processed from a precursor protein in a fashion similar to the maturation pathway of SMR1 and peptide-hormone precursors. Furthermore, as for the QHNPR rat peptide (SEQ ID NO: 8), the excreted QRFSR peptide (SEQ ID NO: 3) seems to be accumulated in the human salivary secretions under different forms, among which the free forms including probably an acetate salt form and the complex forms involving high hydrophobic interactions with salivary PRP-E.

Please amend the paragraph beginning at page 45, line 21, as follows:

EXAMPLE 4 : Synthesis and testing of QRFSR (SEQ ID NO: 3) peptide

The QRFSR peptide (SEQ ID NO: 3) was synthesized and analyzed for its capacity to inhibit the degradation of the physiological NEP substrate, the substance P, *in vitro*, in the experimental model of static incubation of human LNCaP cell membranes. The peptide

QRFSR (SEQ ID NO: 3), inhibited the extra-cellular endoproteolysis of substance P mediated by human NEP expressed at the surface of human prostate epithelial cells. The effective concentration for QRFSR (SEQ ID NO: 3) ranged from 1 to 25 μM, and being half-maximal (IC50) at 11 μM (Figure 5). Surprisingly, but in redundant way with regard to what was observed with rat sialorphin towards the human NEP, the inhibitory efficiency of the QRFSR human peptide (SEQ ID NO: 3) towards the rat renal NEP activity is at least 10-fold lower than that obtained towards the human cell surface NEP (LNCaP). Strikingly, the derivative peptide YQRFSR (SEQ ID NO: 4), which has been synthesized for tritium labeling and immunogenic conjugation for the development of antibody and immunoassay detection system, appeared to exhibit a relatively similar inhibitory efficacy towards both human and rat ecto-endopeptidase activities (Figures 6 and 7).

Please amend the paragraph beginning at page 46, line 4, as follows:

Table : inhibitory potency of natural and derivative human and rat peptides towards both human and rat ectoendopeptidase activities :

Ectoendopeptidase from	Human cells	Rat tissues
QHNPR (SEQ ID NO: 8)	4 to 40 μM	0.4 to 4 μM
QHNP (SEQ ID NO: 9)	undetermined	≥ 50 μM
QRFSR (SEQ ID NO: 3)	2.5 to 25 μM	≥ 100 μM
YQRFSR (SEQ ID NO: 4)	5 to 50 μM	5-75 μM
QRGPR (SEQ ID NO: 10)	≥ 90 μM	undetermined
QRGPRGP (SEQ ID NO: 11)	≥ 90 μM	undetermined

Please amend the paragraph beginning at page 46, line 8, as follows:

Besides, the QRGPR peptide (SEQ ID NO: 10) (20 - 90 μM) which could be potentially maturated from *hPB* gene products, had no effect on substance P endoproteolysis induced by LNCaP human cell membranes; this result lets the inventors to propose that the nature of three central amino acids of the natural NEP-inhibitor pentapeptide (common Q-Nterminal and R-Cterminal) is determining signature for the affinity and/or specifity of their functional interaction with NEP ectoendopeptidase. Furthermore, in spite of the strong primary amino-acid sequence analogy between the rat and human NEP (≠85 %), the inventors observed a relative specificity in the functional interaction of both natural inhibitor-pentapeptides, respectively the rat QHNPR (SEQ ID NO: 8) and human QRFSR (SEQ ID NO: 3). All these results provide evidence for the existence of a conformational specificity in the secondary and tertiary of both ectoenzymes; the crystal structure determination of the binary complex formed with the sialorphin or its derivatives and the human NEP should allow to gain insight into the binding mode of these natural competitive inhibitors.

Please amend the paragraph beginning at page 46, line 23, as follows:

The inventors used the tritiated 3H-YQRFSR peptide (SEQ ID NO: 4) to establish the pharmacokinetic and pharmacodynamic parameters, of this human functional peptidomimetic of rat sialorphin *in vivo* in adult male rat (biodistribution-bioavailability-clearance) as well as to define its metabolism mechanism and turnover *in vivo* and *in vitro,* (Figure 8). The RP-HPLC chromatographic characteristics revealed that :

- the YQRFSR peptide (SEQ ID NO: 4) is not metabolized by human cell membranes containing NEP, indeed 93 % was recovered as intact peptide against 94 % in absence of metabolizing membranes,

- in the same experimental conditions, the YQRFSR peptide (SEQ ID NO: 4) inhibits by 70% the endoproteolysis of substance P by these human cell membranes.

Please amend the paragraph beginning at page 47, line 8, as follows:

Therefore, YQRFSR (SEQ ID NO: 4) is useful for investigating the analgesic activity of the BPLP maturation products in behavioral rat models of acute pain, e.g., the Pin pain test and Formalin test, which have been studied for the functional characterization of the sialorphin *in vivo* (Rougeot et al., 2003).

Please amend the paragraph beginning at page 47, line 13, as follows:

EXAMPLE 5 : Further characterization of QRFSR (SEQ ID NO: 3) peptides *in vitro*

The inhibitory specificity of the QRFSR-peptide (SEQ ID NO: 3) was assessed by measuring the endoproteolysis of substance P (SP) in an *in vitro* enzyme-assay using purified soluble human NEP and human DPPIV (without the N-terminal cytosol and transmembrane segment). Using the selective recombinant hNEP assay, the molecular interaction of human QRFSR-peptide (SEQ ID NO: 3) with hNEP was established, providing direct evidence that the peptide inhibited hNEP activity: as shown on Figure 9, QRFSR-peptide (SEQ ID NO: 3) prevented the NEP mediated-endoproteolysis of SP by 90%; its inhibitory potency was strictly concentration dependent (r^2= 0.99, n=18), ranged from 5 to 50 µM and was half-maximal at 29 ± 1 µM. In contrast, the breakdown of SP by recombinant hDPPIV was not prevented by 25 or 50 µM QRFSR-peptide (SEQ ID NO: 3), indicating that the inhibitory potency of the QRFSR-peptide (SEQ ID NO: 3) on the SP-catabolizing cell surface ectoenzymes *in vitro*, is simply due to its specific interaction with NEP-ectopeptidase. Furthermore, from studies monitoring the *in vivo* metabolism of SP, it appears likely that the

QRFSR-peptide (SEQ ID NO: 3), like rat QHNPR-sialorphin (SEQ ID NO: 8), does not entirely protect endogenous SP from cleavage by the spinal SP-inactivating ectopeptidases, and therefore would not potentiate SP-mediated nociception *in vivo*.

Please amend the paragraph beginning at page 48, line 3, as follows:

Thus, the inhibitory specificity of QRFSR-peptide (SEQ ID NO: 3) was assessed in an enzyme-assay using membrane preparations of recombinant HEK human cells expressing selectively either human membrane-anchored NEP or APN. These transfected-cell models were developed in the laboratory. Membrane amino- and endo-ectopeptidase activities of human cell membranes were assayed *in vitro* by measuring the breakdown of artificial specific fluorogenic substrates, the NEP substrate used was: Mca-R-P-P-G-F-S-A-F-K-(Dnp)-OH (SEQ ID NO: 12) (Mca-BK2) and the APN substrate was: Ala-pNA. Using the selective membrane-anchored hNEP assay, the inventors found that the inhibition by the QRFSR-peptide (SEQ ID NO: 3) of Mca-BK2 endoproteolysis by NEP is concentration dependent (r^2= 0.88, n = 29 determination points) and the effective doses ranged from 5 to 50 µM. Using the selective membrane-anchored hAPN assay, the inventors have demonstrated that QRFSR-peptide (SEQ ID NO: 3) inhibits the Ala-pNA cleavage by hAPN at 10 to 90 µM effective doses (r^2~~2~~ = 0.93, n=22 determination points) (see Figures 10 and 11).

Please amend the paragraph beginning at page 48, line 17, as follows:

Table 1 : Summary of QRFSR (SEQ ID NO: 3) inhibitory effects (IC_{50}) on NEP and APN ectoenzyme activities, *in vitro* and *ex vivo* :

Please amend the paragraph beginning at page 48, line 21, as follows:

These results indicate that the human QRFSR-pentapeptide (SEQ ID NO: 3) is an efficient dual inhibitor of NEP and APN ectopeptidase activities, *in vitro*. Furthermore, owing to the complementary role of NEP and APN in enkephalin inactivation and by analogy with rat sialorphin which exerts a powerful analgesic activity, the combined biological and genomic information accrued led the inventors to propose that the QRFSR-peptide (SEQ ID NO: 3), by inhibiting enkephalin-inactivating NEP-APN ectopeptidases, potentiates enkephalin-dependent antinociceptive mechanisms, *in vivo*.

Please amend the paragraph beginning at page 49, line 7, as follows:

EXAMPLE 6 : Functional characterization of QRFSR (SEQ ID NO: 3) peptide *in vivo*

In spite of the strong primary amino-acid sequence analogy between the rat and human NEP (≠85 %), the inventors observed a relative species-selectivity in the inhibitory potency of both inhibitor-pentapeptides, respectively the rat QHNPR (SEQ ID NO: 8) and human QRFSR (SEQ ID NO: 3). Strikingly, the derivative peptide YQRFSR (SEQ ID NO: 4), which was synthesized for tritium labeling, appeared to exhibit a relatively similar inhibitory efficacy towards both human and rat ectoendopeptidases (range of effective concentrations between 5 and 50 μM). Thus, the antinociceptive potency of the QRFSR-derived peptide (SEQ ID NO: 3) was investigated in the behavioral rat model of acute pain, i.e., the formalin test, which was used for the *in vivo* characterization of rat sialorphin action (Rougeot et al., 2003). Systemic administration of 0.5 and 1 mg/kg YQRFSR-peptide (SEQ ID NO: 4) inhibited the early phase (first 20 min after formalin injection) of paw licking of the formalin-injected hind paw. For instance, it significantly reduced the time spent by treated rats in paw licking from 144 ± 17 s, n= 8 (vehicle) to 97 ± 14 s, n=8 (0.5 mg/kg) (p=0.05) and

to 84 ± 13 s, n=8 (1mg/kg) (p = 0.02 by Dunnett t-Test). Surprisingly, in contrast to rat sialorphin-treated rats, the YQRFSR peptide (SEQ ID NO: 4)-treated rats spent significantly less time in paw licking during the late phase (40 to 60 min after formalin injection) of the formalin test (vehicle-treated rats: 63 ±13 s *vs.* 1mg/kg treated-rats: 9 ± 3 s, p = 0.001). Although less potent than rat sialorphin, in term of effective doses (100-200 µg/kg, *iv*), the QRFSR-derived peptide (SEQ ID NO: 3) seems to be as efficient in its pain-suppressive potency (1 mg/kg, *iv*), as the synthetic mixed NEP-APN inhibitor RB101 (2.5-5mg/kg, *iv*) in the formalin-induced pain model.

Please amend the paragraph beginning at page 49, line 30, as follows:

These data (as presented on Figures 12, 13 and 14) clearly indicate that the YQRFSR-peptide (SEQ ID NO: 4) inhibits nociception induced by acute and long-acting chemical stimuli.

Please amend the paragraph beginning at page 50, line 2, as follows:

Furthermore, the analgesia induced by the QRFSR-derived peptide (SEQ ID NO: 3) in the chemical-evoked pain behaviour is totally reversed in the presence of an opioid receptor antagonist, the nalaxone, which is consistent with an involvement of the endogenous opioidergic pathways in its analgesic effect.

Please delete the originally filed Sequence Listing.

Beginning on new page 63, please insert the attached Substitute Sequence Listing.